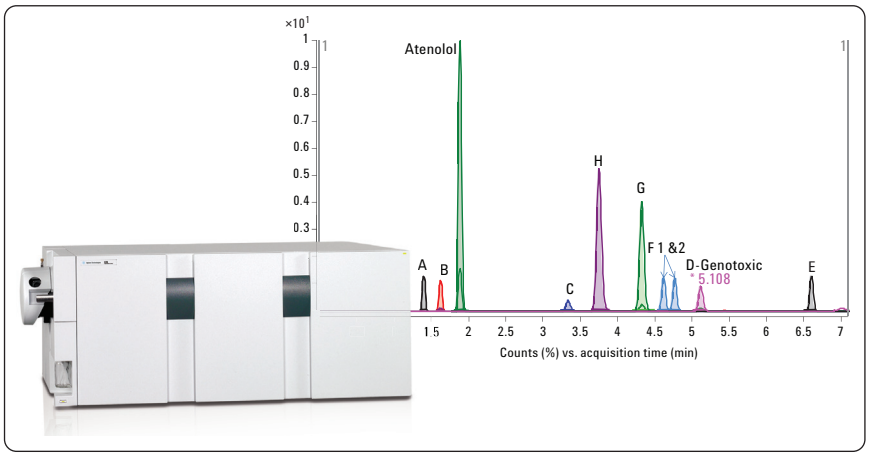
Quantification of genotoxic "Impurity D" in Atenolol by LC/ESI/MS/MS with Agilent 1200 Series RRLC and 6410B Triple Quadrupole LC/MS

Application Note

Manufacturing Process Development



Author

Siji Joseph

Agilent Technologies

Bangalore, India

Abstract

Atenolol is a cardio selective beta blocker. It is used in the treatment of hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. Atenolol may also use in the prophylactic treatment of migraine.[1] Out of eight specific impurities of atenolol (as per European Pharmacopeia) one of them (Impurity D) is a potential genotoxic impurity. Thus sensitivity of the purity-indicating method becomes critical for the quantification of this impurity. This Application Note describes a UV and MS detector chromatographic method for quantification of atenolol impurities. The method developed here is simple, shorter in runtime, with clearly better resolution and higher sensitivity than the existing European and United States Pharmacopeia (EP and USP) methods. Agilent's MassHunter Optimization software was used for quick optimization of MS parameters.

The Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system with UV detection, potential impurity can be quantified in the range of 2.0% to 0.03% with a linearity coefficient of >0.9999 (limit percentage is < 0.25%). Connecting the Agilent 6410B Triple Quadrupole (QQQ) LC/MS detector to an Agilent 1200 Series RRLC system, quantification range can be effectively reduced further down to 5 ppb with a linearity >0.9992. The monitored transition for Impurity D was *m/z* 244.1 to *m/z* 107.

Agilent Technologies

Introduction

The European Directorate for the Quality of Medicines and Health Care (EDQM) has recently emphasized the importance of establishing guidelines for handling potential genotoxic impurities. These guidelines can be used during the revision of monograph specifications. The EP (6.0) lists out eight specific impurities in atenolol, 2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl], designated as Impurities A to H. Impurity D (an alkyl chloro compound) is known to be a potential genotoxic.[2] The molecular structures for atenolol and the eight impurities as per the EP are shown in Figure 1.

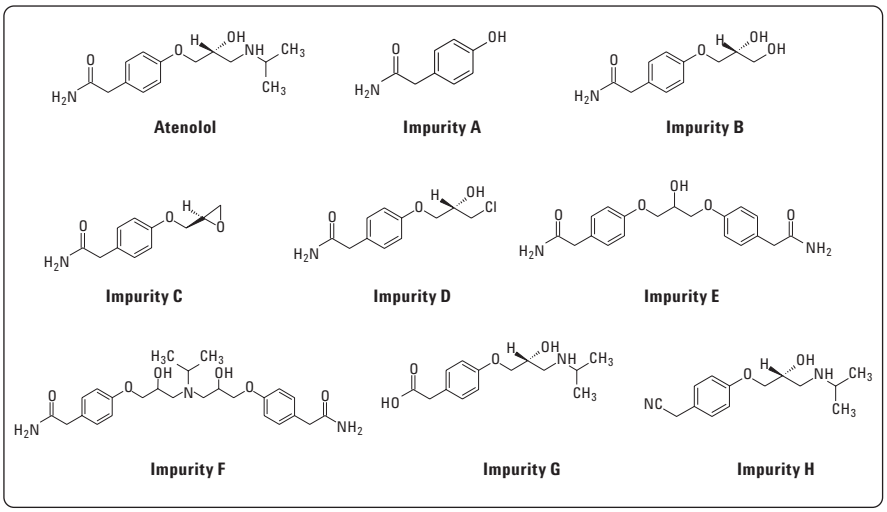


Figure 1
Molecular structures of Atenolol and eight specific impurities as per EP (6.0).

Materials

Atenolol and all the related impurities were purchased from LGC Promochem, Germany. All solvents used in this study were of HPLC grade; acetonitrile was purchased from Labscan and trifluoroacetic acid (TFA) from Fluka. Millipore deionized water was used. All other chemicals used to perform the USP and EP methods were purchased from Sigma Aldrich.

Instrumentation

All LC analyses were performed using the Agilent 1200 SL Series Rapid Resolution (RRLC) system. The system components included an Agilent 1200 Series Binary Pump SL with degasser, Agilent 1200 Series Autosampler SL, and Agilent 1200 Series Thermostatted Column Compartment SL. ESI/MS/MS was performed using Agilent's 6410B triple quadrupole LC/MS system. The Agilent MassHunter Workstation software (version: B.01.04) was used for system control and data acquisition.

Sample preparation

Sample preparation for the LC experiments

Stock solutions of 1000 ppm of each impurity and 2000 ppm of atenolol were prepared separately. Linearity levels of all impurities at a concentration of 20 ppm (level: 8), 10 ppm (level: 7), 5 ppm (level: 6), 2.5 ppm (level: 5), 1.25 ppm (level: 4), 0.625 ppm (level: 3), 0.313 ppm (level: 2), and 0.156 ppm (level: 1) were prepared from this stock solution using the 1 ppm solution of atenolol as a diluent. The exact volume of each impurity stock solution used for the dilution was calculated with respect to individual weight and potency. All the above linearity levels except level 5 were injected three times and level 5 was injected six times (system suitability solution) for the linearity curve.

Sample preparation for ESI/MS/MS experiments

Stock solution concentration of impurities and atenolol for ESI/MS/MS experiments was 1 ppm. This solution was diluted further with 1 ppm atenolol solution to get the lower levels. All the levels were injected five times and the linearity curve was plotted.

LC method development

First, the chromatographic elution of atenolol with all related impurities using the European Pharmacopeia (EP) and United States Pharmacopeia (USP) methods was reproduced for comparison. The USP method[3] was performed using an Agilent ZORBAX Eclipse Plus C18 (4.6 mm id × 250 mm, 5 µm) column and for the EP method an Agilent ZORBAX ODS (4.6 mm id × 150 mm, 5 µm) column was used. The representative chromatograms are shown in Figure 2 (a and b).

The chromatographic methods described in EP as well USP are isocratic and in both cases the buffers are not compatible with mass spectroscopy (MS). For an MS compatible method, methanol containing TFA as modifier was found to give a better resolution for all impurities. TFA facilitated the formation of intense molecular ions without any major adduct. Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 50 mm, 1.8 µm) was used for all chromatographic separations. The LC parameters are shown in Table 1. Premixed solution of mobile phase A & B in the ratio 1:1 was used as the diluent. Gradient time points are as listed in Table 2. A representative LC chromatogram is shown in Figure 2c.

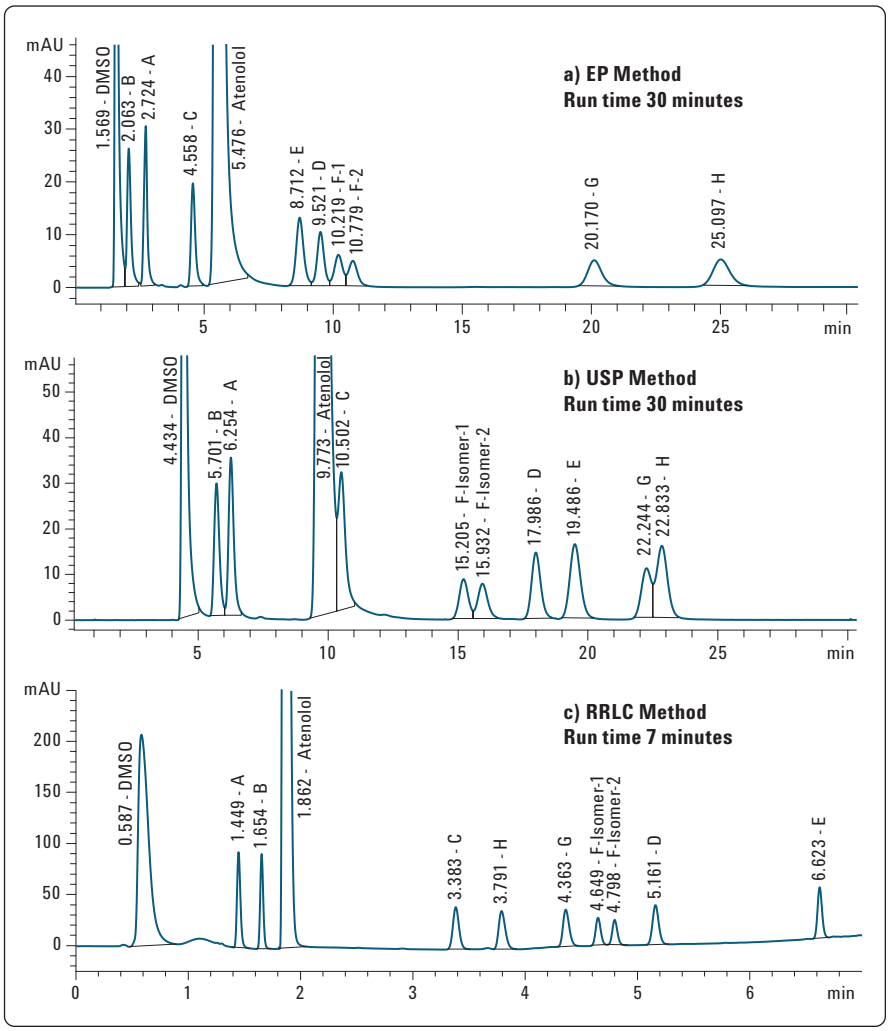


Figure 2
Chromatographic elution pattern for atenolol and eight specific impurities as per (a) EP, (b) USP, (c) RRLC method.

Parameter	Details
Mobile phase A	Water containing 0.1% TFA
Mobile phase B	Methanol with 0.05% TFA
Flow rate	1.5 mL/min
Post run time	2.5 minutes
Injection volume	5 µL
Needle wash mode	Flush port activated for 10 seconds
Column temperature	25 °C
Detection	226 nm

Table 1
LC parameters.

Time (min)	% Mobile phase A	% Mobile phase B
0	95	5
0.1	80	20
2.5	80	20
5.0	70	30
6.5	60	40
7.0	60	40
7.1	95	5

Table 2
Gradient time points used for experiment.

ESI/MS/MS method details

The column eluate was introduced into the electrospray ionization (ESI) source. The nebulizing gas flow rate was set at 13 mL/min, drying gas temperature at 345 °C, the capillary voltage at 4000V, and the nebulizer was at 60 psi.

MS parameters were optimized using the Agilent MassHunter Optimizer software (version: B.01.04). This software automatically optimizes the data acquisition parameters for multiple reaction monitoring (MRM) mode. Each compound at a concentration of 500 ppb was used individually for the optimization. MS parameters obtained from the optimizer software are tabulated in Table 3 (in order of elution). The responses of atenolol and impurities were measured by MRM in the positive ionization mode with corresponding fragmentor voltages and collision energies. All the ions had a dwell time of 200 msec per ion. The elution pattern for atenolol and its impurities are shown in Figure 3.

Compound	Molecular weight	Precursor ion (*m/z*)	Quantifier (*m/z*)	Qualifier (*m/z*)	Fragmentor voltage (V)	Collision energy (V)
Impurity A	151.16	152.1	107	134.1	97	15
Impurity B	225.24	226.1	145	107	107	14
Atenolol	266.33	267.2	145	190	129	26
Impurity C	207.22	208.1	133	178	82	10
Impurity H	248.32	249.1	172	207.1	124	12
Impurity G	267.32	268.1	145.1	190	128	22
Impurity F	473.56	474.3	281.1	145	178	34
Impurity D	243.68	244.1	107	145	111	23
Impurity E	358.38	359.1	107	145	125	47

Table 3
MS optimization parameters for all compounds as per MassHunter Optimizer.

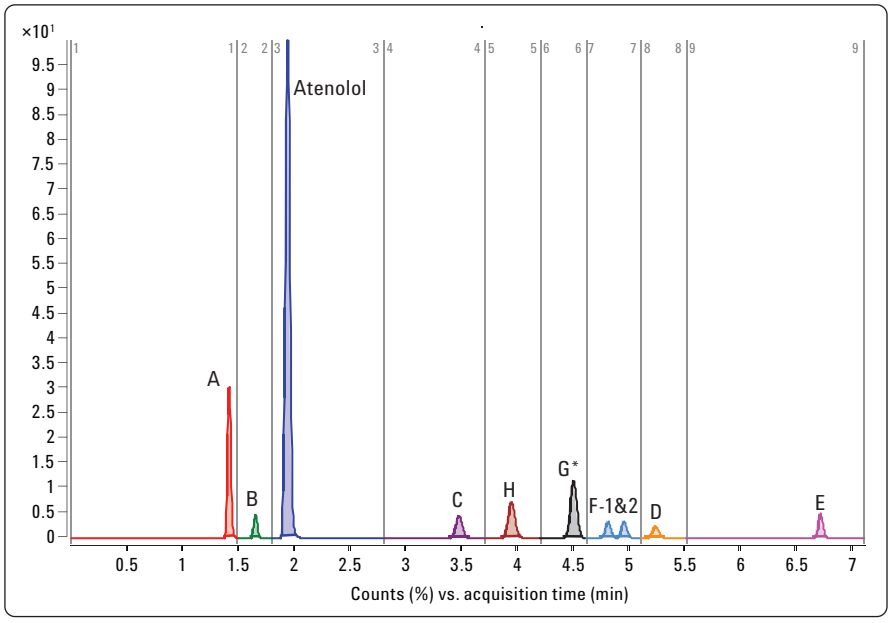


Figure 3
Overlaid MRM ion chromatogram for atenolol and eight specific impurities.

Summary of Results

When compared to the existing EP and USP methods the new method described here has the following advantages:

Shorter runtime:
The elution pattern in the new RRLC method was as shown in Figure 4 which clearly separates all peaks in a runtime of 7 min. Shorter runtimes increase efficiency and productivity.

Better resolution of peaks:
The resolution between Impurity D (Retention time (RT): 9.521) and the first peak Impurity F (RT: 10.219) as per the EP method was 1.27. For the USP method, resolution between Impurity A (RT: 6.254) and Impurity B (RT: 5.701) was 1.52. The resolution between Impurity C (RT: 10.502) and the atenolol peak (RT: 9.773) was 1.41. Resolution between Impurity H (RT: 22.833) and Impurity G (RT: 22.244) was a lower value of 0.70.

In the new method, the observed lowest resolution value is 3.02 (which is between atenolol and Impurity B). Increased resolution of the impurities gives better control for monitoring impurities during the manufacturing process. The peak purity feature in chemstation software confirmed the purity of each peak.

Improved separation of Impurity F isomers:
Observed resolution between Impurity F isomers (RT: 10.219 & 10.779 min) using the EP method was 0.86 and with the USP method it was 1.08 (RT: 15.205 &15.932min). The new method significantly improved this resolution value to 1.77 (Figure 4).

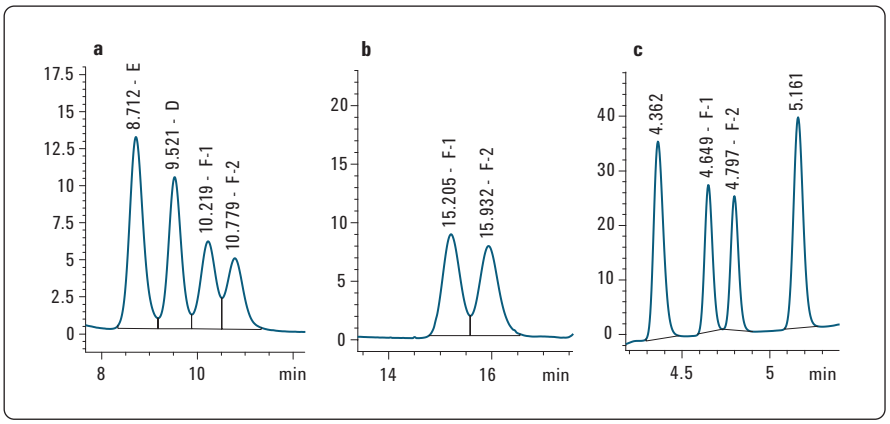


Figure 4
Zoomed view of F-Isomer separation as per (a) EP, (b) USP, (c) Agilent 1200 Series RRLC.

Enhanced sensitivity:
The new RRLC-UV method provides significantly better sensitivity for direct quantification of potential Impurity D in a concentration range of 0.03% (0.315 ng/µL: limit of quantitation (LOQ)) to 2.0% (20 ng/µL). The observed limit of detection (LOD) is 0.0156% (0.156 ng/µL). From a statistical perspective, relative standard deviation (RSD) for retention time at LOD level was 0.022%, and that for the area was 1.992% (n=3). Usage of an ESI/MS/MS detector improved LOQ of potential Impurity D further down to 0.005 ng/µL (5 ppb), whereas the LOD is 3 ppb.

Excellent UV detection Linearity:
The linearity for all impurities from 0.0156% to 2.0% was >0.9999. This proves outstanding linearity capability of the Agilent 1200 Series RRLC detector.

Excellent RSD for retention time and area:
The RSD of retention time and areas was calculated for the system suitability run (impurity concentration level 0.25%, n=6) and are shown in Table 4. The excellent results prove the accuracy and precision of the 1200 Series RRLC injector.

Sample	Area RSD	RT RSD
A	0.201%	0.036%
B	0.247%	0.000%
Atenolol	0.151%	0.028%
C	0.217%	0.046%
H	0.232%	0.037%
G	0.183%	0.082%
F-Isomer-1	0.173%	0.095%
F-Isomer-2	0.131%	0.087%
D	0.186%	0.059%
E	0.153%	0.054%

Table 4.
RT and area RSD for system suitability replicates.

ESI/MS/MS Linearity:
The ESI/MS/MS calibration curves for all impurities from LOQ level to 500 ppb show an excellent MS/MS linearity of >0.99. LOD, LOQ, signal-to-noise ratios (S/N) at LOQ levels and regression coefficient values for each impurity is tabulated in Table 5. The linearity curve for Impurity D is as shown in Figure 5.

MS/MS Recovery:
Accuracy of recovery for all lower levels was within a range of 80% to 120%. The mean recovery of Impurity D was 100.65 ± 9.05% (range 91.6% to 109.7%). These results demonstrate that the method is reliable over a wide concentration range of 5 ppb to 500 ppb atenolol. Impurity D at a concentration of 5 ppb (LOQ) with a signal-to-noise ratio of >11 can be easily quantified using this instrument configuration.

Sample name	LOD (ppb)	LOQ (ppb)	S/N at LOQ	Regression coefficient
Impurity A	<1	1	>23	>0.991
Impurity B	2	4	>10	>0.999
Impurity C	2	3	>12	>0.999
Impurity D (genotoxic)	3	5	>11	>0.999 (Figure 5)
Impurity E	2	3	>10	>0.994
Impurity F	2	4	>12	>0.999
Impurity G	<1	1	>40	>0.995
Impurity H	1	2	>19	>0.992

Table 5
LOD, LOQ, S/N and linearity coefficient values for each impurity.

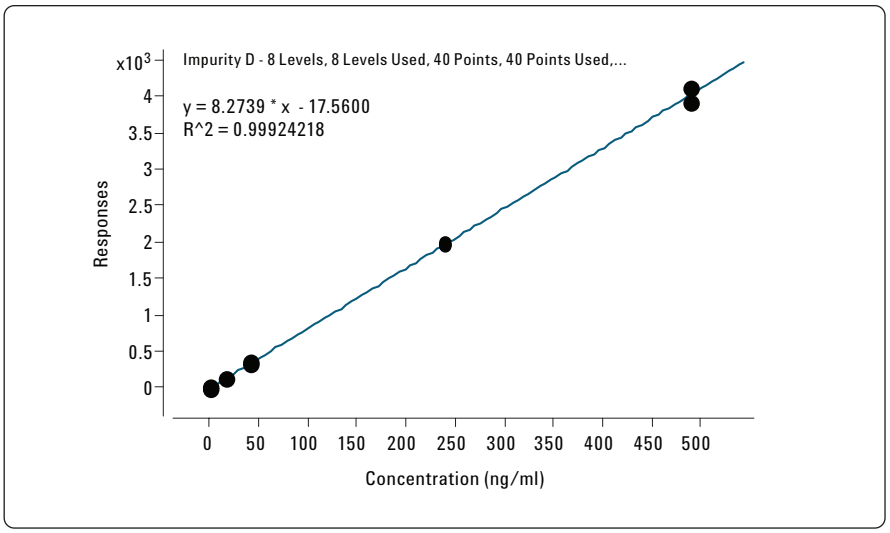


Figure 5
Calibration curve for Impurity D from 5-500 ppb.

Conclusion

The Agilent 1200 Series RRLC system delivers a fast, accurate, sensitive and reliable method for measurement of genotoxic Impurity D in atenolol. This method gives a lower LOD and LOQ with UV detection than existing methods, and meets all the requirements for QA/QC applications. The coupling of a highly sensitive Agilent 6410B Triple Quadrupole LC/MS to an Agilent 1200 Series RRLC system delivers lower LOD and LOQ values which ensures better measure on potential impurities in the final drug.

References

1.
Martindale thirty-fifth edition (**2007**), page 1093

2.
D. P. Elder, et al., *J Pharm Biomed Anal* (**2008**).

3.
USP 29 Asian edition Official monographs (**2008**)/Atenolol, page: 211(3)).

4.
European pharmacopoeia 6.0, Volume 2 (**2008**), pages 1228-1229(4)

www.agilent.com/chem/rrlc

August 1, 2009
Publication Number 5990-4460EN

Agilent Technologies